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Size Doesn't Matter in the Heat Shock Response

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Abstract

Heat Shock Factor 1 (Hsf1) is a transcription factor that is often described as the master regulator of the heat shock response in all eukaryotes. However, due to its essentiality in yeast, Hsf1's contribution to the transcriptome under basal and heat shock conditions was never directly determined. Using a chemical genetics approach that allowed rapid Hsf1 inactivation, my colleagues and I have recently shown that the bulk of the heat shock response is Hsf1-independent. Rather than inducing genes responsible for carrying out the various cellular processes required for adaptation to thermal stress, Hsf1 controls a dedicated set of chaperone protein genes devoted to restoring protein-folding homeostasis. The limited scope of the Hsf1 regulon belies its outsize importance in cellular fitness.

Keywords

Hsf1; heat shock; proteostasis; chaperones; Hsp70; Hsp90

Stress responsive transcription factors (TFs) tend to be inert under basal conditions and only called to action when the cell finds itself in an unfavorable situation (Ho and Gasch, 2015). In happily growing yeast cells, the genes that encode these TFs are dispensable. For example, Hac1, the TF that maintains homeostasis in the endoplasmic reticulum (ER) by activating the unfolded protein response (UPR), can be deleted with negligible fitness costs unless cells are exposed to ER stress. Even Msn2 and Msn4 (Msn2/4) – the TFs that regulate the “general stress response” and are activated by myriad stresses including nutrient depletion, hyperosmotic shock and oxidative stress – can both be deleted without deleterious effects. The exception that proves this rule is Hsf1, the heat shock factor, which is essential under all conditions in yeast. What makes Hsf1 so special?

In our recent work, we sought to answer this question (Solis et al., 2016). As is often the case with essential genes, the key to deciphering the function of Hsf1 was to generate a conditional allele. Since temperature sensitivity was not going to be useful (we needed to be able to inactivate Hsf1 under non-heat shock conditions), we turned to a chemical genetics approach pioneered by the Laemmli lab called “anchor away” (Haruki et al., 2008). The anchor away method allows for the rapid and quantitative nuclear depletion of a protein of interest by hitching it to the large ribosomal subunit via rapamycin-inducible heterodimerization. Ribosomal subunits are assembled in the nucleolus, and then transit via the nucleoplasm to the cytosol, thus generating a large and constant one-way flux out of the nucleus. By appending the FKBP12-rapamycin-binding (FRB) domain onto Hsf1 and a

2xFKBP12 tag onto the ribosomal protein Rpl13a, we were able to achieve complete nuclear depletion of Hsf1 within 10 minutes of rapamycin treatment in unstressed cells. We refer to this strain as “Hsf1-AA”. To ensure the rapamycin had no other effects in the Hsf1-AA strain, we used a genetic background in which the TOR kinase is insensitive to rapamycin (*TOR1-1 fpr1*). We verified that nuclear depletion was tantamount to deletion by showing that the Hsf1-AA cells were not viable in the presence of rapamycin and that expression of a second, untagged copy of Hsf1 could rescue growth. Having established the ability to conditionally inactivate Hsf1 in a matter of minutes, we proceeded to assess the immediate fallout of a loss of Hsf1.

We hypothesized that Hsf1 is essential because it drives coordinated expression of chaperone and co-chaperone genes (Johnson et al., 2014) required to maintain protein homeostasis (proteostasis) under all conditions – not just heat shock or other proteotoxic stress conditions. Supporting this notion, it has long been known that Hsf1 binds to target gene promoters under non-heat shock conditions (Erkine et al., 1999; Gross et al., 1990). Indeed, when we anchored away Hsf1, we observed massive protein aggregation accompanied by a decrease in the levels of the Hsp70 and Hsp90 chaperones. To determine the genome-wide effects of an acute loss of Hsf1, we performed two transcriptomic assays following rapamycin addition: native elongating transcript sequencing (NET-seq) to measure active transcription and RNA-seq to measure total transcript levels. A previous report had suggested that Hsf1 regulates upwards of 3% of yeast genes (Hahn et al., 2004), so we expected many transcripts to change upon Hsf1 nuclear depletion. Strikingly, however, only 18 genes showed both reduced transcription and total mRNA levels following rapamycin treatment under basal conditions (Figure 1A). All of these 18 Hsf1-dependent genes (HDGs) showed strong Hsf1 binding peaks in their promoters as determined by ChIP-seq, and all but one HDG encodes a chaperone or other proteostasis-related factor.

If driving basal expression of HDGs is the essential function of Hsf1, then expressing these genes from Hsf1-independent promoters should rescue growth in the presence of rapamycin. Indeed, that was the case. But even more remarkably, we were able to whittle down the set of HDGs to only *two genes*. Simultaneous expression of *SSA2* and *HSC82* – which encode the Hsp70 and Hsp90 chaperones – from Hsf1-independent promoters was sufficient to support yeast cell growth with Hsf1 anchored away. Thus, Hsf1 is indispensable because it is required for the expression of the Hsp70 and Hsp90 chaperones. More broadly, this finding indicates that chaperones are not just stress response factors; they are housekeeping genes required to maintain eukaryotic proteostasis under all conditions.

While Hsf1 is only required for expression of 18 genes under basal conditions, hundreds of genes are induced by heat shock. Does Hsf1 dramatically expand its suite of target genes during heat shock? The short answer is no: Hsf1 maintains and upregulates expression of its dedicated regulon rather than dramatically expanding its target gene repertoire, though it does induce a few more genes only during heat shock. Unfortunately, we were not able to rigorously determine the precise number of heat shock-dependent HDGs using the same strategy we used under non-heat shock conditions. While several hundred genes showed both reduced NET-seq and RNA-seq levels following a 30-minute heat shock in rapamycin-treated cells, the vast majority of these genes were *already down-regulated* during heat shock

in the presence of Hsf1, and were down-regulated *even more* in the absence of Hsf1 function. Predominately encoding ribosomal proteins and biogenesis factors, these genes did not have Hsf1 binding peaks in their promoters. Thus, we attribute the bulk of these changes to secondary effects rather than the direct result of Hsf1 nuclear depletion. Future analysis of earlier heat shock time points should help to resolve this issue. Still, if we restrict our analysis to genes 1) induced at least twofold by heat shock in the presence of Hsf1, 2) that show strong Hsf1 peaks in their promoters during heat shock, and 3) are induced at least twofold less when Hsf1 is anchored away, we get an additional three HDGs: *HSP10*, *HSP26* and *SSA4* (*HSP60* and *SSA3* did not show binding peaks but fulfilled the other criteria). Using another chemical genetics approach, we were able to demonstrate that the vast majority of the genes induced by heat shock are driven by the general stress response TFs Msn2/4, not by Hsf1. Taken together, we find that Hsf1 is not the global regulator of the heat shock response, but rather controls a small module dedicated to protein folding under both basal and heat shock conditions.

Although yeast Hsf1 is essential, its mammalian homolog is not. The *hsf1*^{-/-} knock out mouse is viable and shows no reduction in the basal expression of chaperone proteins (Xiao et al., 1999). Consistent with this result, we generated two independent *hsf1*^{-/-} lines in mouse embryonic stem cells (mESCs) and fibroblasts (MEFs) using CRISPR/Cas9 and found no more significant differences in basal gene expression than we would expect to find by random chance. Most importantly, the genes encoding Hsp70 and Hsp90 remained very highly expressed in the absence of HSF1. However, upon heat shock, both the mESCs and MEFs failed to induce a small set of chaperone protein genes that was remarkably similar to the HDGs defined in yeast. However, as in yeast, the majority of the genes induced by heat shock were Hsf1-independent. This result was independently confirmed in an elegant study from John Lis's laboratory using the precision nuclear run-on sequencing (PRO-seq) method they pioneered to measure transcription across the genome in wild type and *hsf1*^{-/-} MEFs (Mahat et al., 2016). Thus, the compact, proteostasis-specific HSF1 regulon has been conserved throughout the eukaryotic lineage while the regulation of basal and inducible expression has been decoupled in mammalian cells. Although we do not yet know what does control basal chaperone expression in mammalian cells, we speculate that chaperones are co-regulated with the translation machinery to coordinate protein synthesis and protein folding.

The most perplexing result of our study was the observation that yeast Hsf1 binds to the promoters of dozens of genes whose transcription is unaffected by its nuclear depletion. In particular genes like *ICY2*, *UBI4*, and *ZPR1* – genes that are highly expressed, have canonical Hsf1 binding motifs and have been shown in several studies to have Hsf1 bound in their promoters (Hahn et al., 2004; Kim and Gross, 2013) – show at most modestly reduced expression levels when Hsf1 is anchored away (Figure 1B). And genes like *HSP12*, *HSP26* and *KAR2*, which also have known Hsf1 binding sites in their promoters, remain robustly induced by heat shock in the absence of nuclear Hsf1 (Figure 1B). This leads to the rather unsatisfying conclusion that Hsf1 binding is not required to drive transcription of these genes. Is Hsf1 binding inertly or might it have another role besides simply recruiting the transcriptional machinery? Recent literature hints at two other possible functions for Hsf1 binding: directing mRNA modifications and privileging transcripts for efficient translation.

It was recently reported that some mRNAs contain a post-transcriptional modification, pseudouridylation, that was previously thought to be restricted to non-coding, ribosomal and transfer RNAs (Carlile et al., 2014; Schwartz et al., 2014). Intriguingly, Schwartz et al. found that this modification was induced in several HDG mRNAs during heat shock. The functional consequence of these modifications are unclear, but they may promote mRNA stability. Perhaps Hsf1 binding recruits the pseudouridylyase Pus7 to stabilize these messages. In another recent study, Zid and O'Shea found that the presence of Hsf1 binding sites in gene promoters privileged mRNAs for efficient translation during glucose depletion (Zid and O'Shea, 2014). It has yet to be shown whether this also happens during heat shock or is Hsf1-dependent, but it is intriguing nonetheless. In addition to activating transcription, Hsf1 may license mRNAs for modification and translation to enhance gene expression post-transcriptionally.

In summary, we found that “heat shock factor” is a misleading description for the role that Hsf1 plays in yeast cells. First, Hsf1 is not merely a stress-responsive transcription factor like Msn2/4 or Hac1. Hsf1 is active in unstressed yeast cells and essential because it drives a housekeeping program required for proteostasis. And second, Hsf1 is not the master regulator of the heat shock response. The vast majority of genes induced by thermal stress are controlled by Msn2/4 and are Hsf1-independent. Despite its compact size, the highly conserved and dedicated Hsf1 regulon is a core eukaryotic module of the utmost importance. As the saying goes, it's not the size that matters, it's what you do with it.

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Figure 1. Hsf1-dependent genes (HDGs) and Hsf1-independent genes (non-HDGs)

A. mRNA expression levels (FKPM) measured by RNA-seq (taken from Solís et al.) for the 18 HDGs in the presence or absence of rapamycin (rapa) to anchor away Hsf1, under basal conditions (30°C) and following a 30 minute heat shock (39°C). *SSA1* and *SSA2* cannot be distinguished by short-read sequencing and are merged (*SSA1/2*).

B. As in (A), but for genes that did not pass all three filters to be defined as HDGs. All of these genes show at least one of the following: reduced basal or heat shock expression as measured by RNA-seq or NET-seq following Hsf1 nuclear depletion or Hsf1 promoter binding as measured by ChIP-seq under basal or heat shock conditions. The genes highlighted in blue are discussed in the text.